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A NOVEL SULFOTRANSFERASE SULFATES TYROSINE-CONTAINING PEPTIDES AND PROTEINS

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The novel sulfotransferase (M.W. 315 kDa) obtained from *Eubacterium* A-44 catalyzed the sulfation of tyrosine residues of peptides and proteins such as kyotorphin, enkephalin, cholecystokinin-8 (non-sulfated form), trypsin inhibitor and insulin. Also, the enzyme sulfated tyrosine residues of protein fractions purified from *Eubacterium* A-44. © 1986 Academic Press, Inc.

Among various posttranslational modifications of proteins (1-3), protein phosphorylation has been extensively studied. Phosphorylation of tyrosine residues has been implied in regulatory events such as cell transformation and hormone-induced cell growth (4-8). However, recent studies have shown that tyrosine-O-sulfate residues occur in a large number of secretary proteins, only some of which, such as immunoglobulin G and fibronectin, have been identified (9-13) and the role of tyrosine sulfation is generally not yet understood. Also, very little is known about the enzyme(s) responsible for this posttranslatioal modification. We discovered a novel type of arylsulfotransferase obtained from the human intestinal bacterium, *Eubacterium* A-44 (14), and report here the properties of the enzyme, which catalyzes the sulfate transfer reaction to tyrosine residue(s) of peptides and proteins, and the role of the enzyme in *Eubacterium* A-44.

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MATERIALS AND METHODS

Materials. Insulin, trypsin inhibitor, albumin and p-nitrophenyl sulfate (PNS) were purchased from Sigma Chem. Co. Tyrosine methylester and tyramine were from Nakarai Chem. Ltd. Kyotorphin was from Funakoshi Chem. Co. (Leu)-enkephalin and cholecystokinin-8 (non-sulfated form) (CCK-8-NS) were kindly donated from Dr. T. Morikawa, Fuji Chemical Industries, Ltd. (^{35}S) PNS was synthesized according to the previous method (15).

Enzyme activity assay. Sulfotransferase activity was determined by incubation for 15 min at 37°C of the reaction mixture containing 0.03 ml of 50 mM (^{35}S) PNS or cold PNS, 0.2 ml of 1 mM acceptor (except 0.1 mM albumin), 0.18 ml of enzyme (specific activity 13.7 units/mg protein) purified by the previous method (stage 3) (15), 0.02 ml of 0.25 mM $MgCl_2$ and 0.2 ml of 0.1 M glycine-NaOH buffer, pH 8.5.

Determination of protein. Protein was determined by the method of Lowry et al (16) with bovine serum albumin as standard.

Purification of a protein fraction and its sulfation. Cultivated *Eubacterium* A-44 was collected, washed with saline, sonicated, centrifugated and applied to DEAE-cellulose column chromatography equilibrated with 0.1 M acetate buffter, pH 5.7. And then the column was eluted with 0.1 M acetate buffer, pH 5.7 containing 0.15 M KCl. The eluted fraction (free of sulfotransferase activity) was concentrated by ultrafiltration through a UM-10 membrane. The concentrated solution was dialyzed against 0.1 M glycine-NaOH buffer, pH 8.5, overnight (9.49 mg protein/ml).

The sulfation reaction was performed for 100 min at 37°C under the following conditions: The reaction mixture (total volume 1.2 ml) contained 0.38 ml of the purified protein fraction, 0.38 ml of 0.1 M glycine-NaOH buffer, pH 8.5, and 0.02 ml of 0.25 M $MgCl_2$ in the presence of (a) 0.38 ml of an enzyme solution (total activity 2.66 units, sp. act. 13.7 units/mg protein) and 0.04 ml of 50 mM (^{35}S)PNS (3.1×10^6 CPM/mg), (b) 0.38 ml of the enzyme solution and 0.04 ml of 50 mM (^{35}S)Na_2SO_4 (1.5×10^7 CPM/mg), (c) 0.38 ml of the enzyme solution previously inactivated by heating at 100°C for 10 min and 0.04 ml of 50 mM (^{35}S)PNS and (d) 0.04 ml of 50 mM (^{35}S)PNS and 0.38 ml of 0.1 M glycine-NaOH buffer, pH 8.5. The reaction mixture was applied to Sephadex G-50 coarse column chromatography at 4°C, and the column eluted with 0.1 M Tris-HCl buffer, pH 7.0 (fraction volume, 2 ml). The radioactivity for the eluted fractions was determined with a liquid scintillation counter.

RESULTS AND DISCUSSIONS

The novel bacterial sulfotransferase differed from the known mammalian enzymes in the substrate specificity (14,15). The present enzyme catalyzed the transfer reaction of a sulfate group from phenolic sulfate esters but not from 3'-phosphoadenosine 5'-phosphosulfate(PAPS) to another phenolic compound. We studied acceptor substrate specificity for some

Table I. The Specificity of Acceptor Substrate

Substrates	Activity[a] (nmol/min/mg protein)
Tyrosine methylester	3640
Tyramine	3600
Kyotorphin	35.3
(Leu)-enkephalin	218.2
Cholecystokinin-8-nonsulfate(CCK-8-NS)	8.1
Insulin	0.12[b]
Trypsin inhibitor	0.24[b]
Albumin	0.07[b]

a Average value of triplicate determinations.
b The reaction mixture was incubated for 3 hr.

physiologically active peptides and proteins using PNS as a donor (Table I). Although the optimal pH for each acceptor substrate was different, (Leu)-enkephalin whose tyrosine is at the N-terminal position was most effective, followed by kyotorphin, CCK-8-NS and trypsin inhibitor. Trypsin inhibitor was a better substrate than insulin and albumin, although the molecular weight of insulin is lower than trypsin inhibitor and albumin contains more tyrosine residues. Thus, buried tyrosine residues of proteins were hardly sulfated by this novel sulfotransferase. These results suggest that physiologically active peptides and proteins containing tyrosine at the active site may be regulated by the enzyme present in *Eubacterium* A-44.

The enzyme activity was increased approximately ten fold by adding the donor substrate to the culture media, but not by inorganic sulfate or by an acceptor substrate. In addition, when the sulfotransferase-free protein fraction from *Eubacterium* A-44 was incubated with the novel sulfotransferase or its heat-inactivated preparation, in the presence of (^{35}S)PNS or (^{35}S)Na_2SO_4, and the reaction mixture fractionated by Sephadex G-50 coarse chromatography, sulfation of the protein fraction was observed only in the reaction mixture containing active enzyme and PNS (Fig. 1). When, alkaline hydrolysates of the sulfated

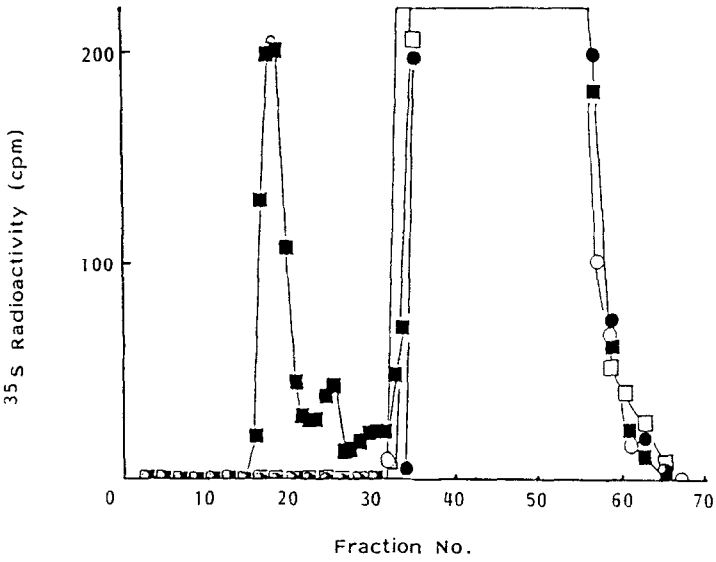

Fig. 1. Sulfation of the purified protein fraction by the novel sulfotransferase. The reaction mixture contained the protein fraction, glycine-NaOH buffer and $MgCl_2$ in the presence of (a, ■) active enzyme and (^{35}S)PNS, (b, □) the enzyme and (^{35}S)Na_2SO_4, (c, ●) the inactivated enzyme and (^{35}S)PNS, and (d, ○) (^{35}S)PNS only. The reaction conditions were described in Text.

protein fractions (No. 17-20) of Sephadex G-50 coarse chromatography were analyzed by cellulose plate electrophoresis (14), an intense radioactive spot coincided with that of authentic tyrosine-O-sulfate (data not shown).

It will be of interest to identify the proteins which are sulfated and to determine the physiological role of the novel tyrosine-protein sulfotransferase.

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